Sarcolemmal $K_{ATP}$ Channel Triggers Opioid-Induced Delayed Cardioprotection in the Rat

Hemal H. Patel, Anna K. Hsu, Jason N. Peart, Garrett J. Gross

Recently, the involvement of sarcolemmal $K_{ATP}$ (sarc$K_{ATP}$) channels in ischemic and pharmacological preconditioning (IPC and PPC) has been minimized by numerous studies suggesting a primary role for mitochondrial $K_{ATP}$ (mito$K_{ATP}$) channels in early and delayed cardioprotection. Although the mito$K_{ATP}$ channel has clearly been shown to be a distal effector of delayed IPC and PPC, studies implicating it as a trigger of protection in delayed IPC are lacking. Accordingly, we characterized the role of cardiac $K_{ATP}$ channels as triggers or distal effectors of delayed cardioprotection induced by opioids in rats, and the data suggest that the sarc$K_{ATP}$ channel triggers and that the mito$K_{ATP}$ channel is a distal effector of opioid-induced delayed cardioprotection.

Protection from myocardial infarction via ischemic preconditioning (IPC) has been described to be a biphasic event. The early phase occurs immediately after IPC and lasts 1 to 3 hours; the late phase of protection is seen 12 to 24 hours after the initial stimulus and lasts up to 72 hours. We have previously shown that $\delta$ opioid agonists induce a delayed cardioprotective effect that appears to be mediated by a burst of reactive oxygen species (ROS).

In recent investigations, the mitochondrial ATP-dependent $K^-$ (mito$K_{ATP}$) channel has received considerable attention as being the trigger of early IPC. Studies in the isolated rabbit heart and in isolated myocytes have suggested that this channel contributes to protective signals via a redox-sensitive mechanism. Similarly, data suggest that the mito$K_{ATP}$ channel could be either a trigger that induces the expression of proteins or a distal effector in delayed IPC. However, little information is available on the triggering role of the sarcolemmal $K_{ATP}$ (sarc$K_{ATP}$) channel in delayed IPC.

Therefore, we investigated the role of the sarc$K_{ATP}$ channel as a trigger or distal effector of delayed cardioprotection induced by $\delta$ opioid agonists. We present evidence suggesting that the sarc$K_{ATP}$ channel functions as the trigger and the mito$K_{ATP}$ channel as a distal effector in opioid-induced delayed PC.

Materials and Methods

This study was performed in accordance with the guidelines of the Animal Care Committee of the Medical College of Wisconsin, which is accredited by the American Association of Laboratory Animal Care.

Study Groups and Experimental Protocols

Male Sprague-Dawley rats (250 to 300 g) were obtained from Charles River Laboratories, Wilmington, Mass, and randomly divided into groups and subjected to pretreatment with SNC-121 (0.1 mg/kg, IV), a $\delta$ opioid agonist, followed by a 24-hour recovery period. Selective and nonselective $K_{ATP}$ channel blockers were administered intravenously either with opioid pretreatment or after the recovery period just before index ischemia. All rats underwent 30 minutes of index ischemia followed by 2 hours of reperfusion.

General Surgical Procedure and Infarct Determination

General surgical procedures and infarct determination were performed as described previously. Briefly, rats were anesthetized, vessels cannulated for delivery of drugs and blood pressure measurements, and a tracheotomy performed for artificial ventilation. Subsequently a left thoracotomy was performed at the fifth intercostal space, and a pericardiotomy was performed by adjustment of the left atrial appendage to locate the left coronary artery. A ligature was passed below the left descending vein and coronary artery from the area immediately below the left atrial appendage to the right portion of the left ventricle. The ends of the suture were threaded through a propylene tube to form a snare. Occlusion was elicited by clamping the snare onto the epicardial surface using a hemostat.

After 2 hours of reperfusion, the coronary artery was again occluded. The area at risk (AAR) was determined by negative staining. The heart was excised, and the left ventricle was separated from the remaining tissue and cut into thin cross-sectional pieces. The normal area and AAR were separated and placed in different vials containing 1% 2,3,5-triphenyltetrazolium chloride (TTC) in 100 mmol/L phosphate buffer (pH 7.4). Tissues were fixed overnight in 10% formaldehyde, and the infarcted tissue was dissected from the AAR using a dissecting microscope. In 9 additional rats (5 treated with SNC only and 4 treated with SNC+HMR-1098), epicardial monophasic action potential duration at 50% repolarization (APD$_{50}$) was determined by a bipolar electrode (EP Technologies) as previously described. The average of 10 consecutive arrhythmia-free readings was used to calculate APD$_{50}$.

Statistical Measurements

All values are expressed as mean SEM. For the hemodynamic data, left ventricle mass, AAR, infarct size, and APD, statistical significance was determined by performing a one-way ANOVA with Bonferroni’s multiple-comparison test as the post hoc test. Significance was set at $P<0.05$.

Results and Discussion

Rats were simultaneously pretreated with SNC and glibenclamide (3 mg/kg), a nonselective $K_{ATP}$ channel blocker, HMR-1098 (6 mg/kg), a selective sarc$K_{ATP}$ channel blocker, or 5-hydroxydecanoic acid (5-HD; 10 mg/kg), a selective mito$K_{ATP}$ channel blocker, before undergoing a 24-hour recovery period.
None of the \(K_{\text{ATP}}\) channel blockers given alone resulted in an infarct different from control; however, the delayed cardioprotective effect of SNC (29±2% versus 58±1%, \(P<0.001\)) was attenuated by simultaneous treatment with glibenclamide (46±4%, \(P<0.001\)) or HMR-1098 (51±5%, \(P<0.001\)) but not with 5-HD (35±3%) (Figure 1). In addition, SNC shortened APD\(_{50}\) from 61±9 to 31±4 ms (\(P<0.01\)) but not with 5-HD (35±3%) (Figure 1). In addition, SNC shortened APD\(_{50}\) from 61±9 to 31±4 ms (\(P<0.01\)).

Mechanistically, our data suggest that SNC may be facilitating opening of the sarc\(K_{\text{ATP}}\) channel has been associated with opening of the sarc\(K_{\text{ATP}}\) channel as a trigger. It is not known how opening the sarc\(K_{\text{ATP}}\) channel produces a delayed cardioprotective effect in rats; however, it is feasible that ROS are involved.

Opening of the sarc\(K_{\text{ATP}}\) channel has been associated with shortening of the APD. Hyperpolarization of the cell, decreased calcium entry, and preserved ATP production. Mechanistically, our data suggest that SNC may be facilitating the trigger phase via shortening of APD because HMR-1098 blocked the APD shortening and cardioprotective effect of SNC. We have shown in previous studies that the trigger phase of early IPC or pharmacological preconditioning (PPC) is also linked to the generation of an early burst of ROS, the source and timing of which are presently unknown. The possibility exists that ROS may modulate sarc\(K_{\text{ATP}}\) channel activity to trigger delayed cardioprotection. It has been suggested that there is crosstalk between the mito\(K_{\text{ATP}}\) and sarc\(K_{\text{ATP}}\) channels in that ATP consumption by mitochondria activates sarc\(K_{\text{ATP}}\) channels. It is possible that opioids interact with mitochondria and modify sarc\(K_{\text{ATP}}\) channel activity by modulation of redox-sensitive or metabolic mechanisms that trigger delayed cardioprotection. Interestingly, it was shown in a sarc\(K_{\text{ATP}}\) channel knockout mouse, that IPC was absent. These data and the present results suggest that the sarc\(K_{\text{ATP}}\) channel needs to be further evaluated as a possible trigger in other models of delayed cardioprotection.

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References


Key Words: delayed preconditioning • opioids • trigger • sarcolemmal K\(_{ATP} \) • mitochondrial K\(_{ATP} \)
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